ESTIMATION OF SERUM LEVELS OF VITAMIN D AND CALCIUM IN RENAL DISEASE PATIENTS IN EDO STATE, SOUTH-SOUTH ,NIGERIA

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ABSTRACT

Background: Abnormalities in kidney function and/or structure that persist for longer than three months are considered chronic kidney disease. End-stage renal disease, often known as kidney failure, is a medical condition in which the kidneys are unable to remove waste products from the blood. This research focused on assessing vitamin D and calcium levels in patients with renal disease at Central Hospital in Benin City, Nigeria.

Method: Among 110 initially recruited patients, 30 opted out due to religious beliefs, and 48 due to undisclosed information, leaving 46 participants (32 with renal disease, 14 healthy). Using ELISA and spectrophotometric methods, vitamin D and calcium levels were measured. SPSS (IBM) version 23.0 was used to analyze the collected data.

Results: Patients' mean age was 50.59±14.88 years, with a prevalence of smoking (59.4%) and alcohol consumption (84.4%). Serum calcium levels were lower but not statistically significant ($p > 0.05$), while vitamin D levels were significantly below normal ($p < 0.05$). Chronic renal disease exhibited notably lower calcium and vitamin D levels than acute renal disease ($p < 0.05$). Alcohol use correlated with decreased serum vitamin D levels.

Conclusion: Patients with renal disease displayed significantly lower vitamin D levels, potentially contributing to impaired intestinal calcium absorption and subsequent mineral bone issues

Keywords: Renal illness, low vitamin levels, vitamin supplements, uremia, and glomerular filtration rate

INTRODUCTION

Chronic kidney failure, characterized by persistent abnormalities in kidney function, is a growing global health concern (BDA, 2022). When kidney function drops to 15% or less, it leads to kidney failure (BDA, 2022). Symptoms include fatigue, nausea, loss of appetite, disorientation, and leg swelling (NIDDKD, 2017).

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Chronic kidney failure is often linked to conditions like diabetes and untreated high blood pressure ([NIDDKD, 2012), with polycystic kidney disease and certain medications also identified as causes (Kes, 2011). The progression of chronic kidney disease (CKD) is associated with complications such as uremia, high blood potassium, and volume overload, leading to heart disease, high blood pressure, and anemia (Deluca, 2014 and Gois, 2017).

Vitamin D deficiency is common in CKD patients and has implications beyond calcium regulation (Fraser, 1970; Holick, 2004 and Townsend, 2005). The narrative highlights the synthesis of vitamin D in the skin and its forms vitamin D2 and vitamin D3 (VD2 and VD3), as well as its metabolism and transport (Holick, 1977; Deluca, 2014 and Gois, 2017). Vitamin D deficiency is associated with an increased risk of death from cardiovascular disease in CKD patients (Holick, 2004 and Townsend, 2005). There are between 5 and 7 million people with kidney failure who need renal replacement therapy (Zhang, 2019), and the estimated incidence of CKD worldwide is 13.4%. Acute kidney injury, in contrast to chronic kidney disease, frequently leads to kidney recovery, allowing the individual with AKI to lead a normal life again. Patients with acute kidney damage have an increased chance of having renal failure in the future and require supportive care while their kidneys heal (Silva, 2017). As a result, vitamin D (VD's) significance in biology extends far beyond the maintenance of normal levels of calcium and phosphate. This is one of the first studies of its kind to examine the serum vitamin D levels of cigarette and alcohol consumers in patients with CKD who are on the verge of starting dialysis in Benin City.

MATERIALS AND METHODS

Study Population and Design

Patients with renal illness were tested for vitamin D and calcium levels in this case-control study. One hundred ten (110) patients with known renal disease were recruited for the study using our sample size formula; however, n = 30 (opted out of the study on religion belief) and n = 48 (opted out of the study due to undisclosed information necessary for the study), so we end up using forty-six (46) patients, consisting of thirty-two (32) patients with renal disease and fourteen (14) apparently healthy subjects. Patients with renal illness from Central Hospital in Benin City, Edo State, were the study participants, and residents of Benin City served as the comparison group. Benin City, the state capital of Edo, was the location of this investigation. Edo State had a population of 3,233,366 in the 2006 census and is expected to have a population of 4,461,137 in 2019(NPC, 2022). Benin City, Edo State's capital and largest city, is projected to have a population of 1,841,000 in 2022. Benin City is found at coordinates 6.3^0 N and 5.6^0 E.

Inclusion and Exclusion criteria

Males and females over the age of 18 who visited Central Hospital in Benin City with renal illness and gave informed consent were included in the study. In order to collect data, a standardized and structured questionnaire was given to participants who gave their informed consent.

Sample Collection

Each participant had 5 mL of fasting blood drawn from their ante-cubital vein with a sterile needle and syringe under aseptic conditions and without the use of a tourniquet. The blood sample was placed in sterile container. Serum was separated from the clot by centrifuging the sample at 4000 rpm for 5 minutes after it had been left undisturbed to clot for a few minutes. The serum was transferred to a new,marked container. Before analysis could be performed, the serum samples were kept at -20^0 degrees Celsius.

The kidney status classification data were obtained from the patient case files (acute and chronic kidney disease).

Sample Size Determination

Following this formula, we were able to determine an adequate sample size.

$N = z^2 * p (1-p) /d2$

Where N is the minimum sample size and z is the 95% confidence interval (often 1.96).

p = prevalence of the renal disease of interest, estimated from the literature to be 7.8% (Okwuono *et al*., 2017)

D = 5% (or 0.05) of the standard deviation from the true value of a variable.

N = 1.922 * 0.078 (1- 0.078)/0.0025 =110.

Due to patients' reluctance to volunteer their personal health information and sign consent forms, we were only able to study 32 people with renal disorders and 14 healthy controls.

Ethical Approval

The Benin City, Edo State Ministry of Health provided the study's ethical clearance (HA. 737/011, dated April 10, 2020).

Methods

The level of vitamin D and calcium (25-hydroxy (25-OH) were measured using ELISA(Calbiotech kit,USA) and spectrophotometric technique respectively.

Statistical Analysis

Statistical information was provided as percentages for categorical data and as continuous variables for descriptive data. Two groups were compared using an independent samples t-test, and more than two groups were compared using a one-way analysis of. Our cutoff for statistical significance was $P<0.05$. SPSS (Windows version 23.0) was used for all statistical analyses

RESULTS

Table 1 outlined the socio-demographic characteristics and health behaveors of 32 patients with renal diseases and 14 healthy controls. Patients with renal diseases had a significantly higher mean age (50.59 years) compared to healthy controls (41.07 years) ($p = 0.041$). The control group predominantly consisted of individuals aged 20-39 and 40-59 years, males, married, employed, and from Edo state. Patients with renal diseases were mainly in the 40-59 age range, male, married, employed, and from Edo state. The majority of the control group did not smoke, drink alcohol, were exposed to sunlight daily, and did not use dietary supplements. Among patients with renal diseases, a significant portion did not smoke, refrained from alcohol consumption, were daily exposed to sunlight, and mostly did not use dietary supplements. Smoking and alcohol consumption patterns among patients with renal diseases varied, with implications for further investigation into lifestyle factors influencing renal health.

Table 2 outlined clinical characteristics of patients with renal diseases, indicating that 56.2% suffer from acute renal disease, while 43.8% have chronic renal conditions. Of the patients, 59.4% were on medications, with 52.6% on medication for $\geq$ 1 month and 47.4% for less than one month. Regarding limb movement, 71.9% reported no difficulties, while 28.1% experienced some movement challenges.

In Table 3, mean serum levels of calcium and vitamin D were compared between patients with renal diseases and healthy controls. An independent sample t-test revealed no significant difference ($p = 0.273$) in mean calcium levels between patients with renal diseases (1.96 ± 0.50) and the control group (2.13 ± 0.37). However, the mean vitamin D level was significantly lower ($p = 0.006$) in patients with renal diseases (46.65 ± 29.69) compared to the control group (71.47 ± 19.27). This highlights a potential deficiency in vitamin D among patients with renal diseases, warranting further exploration of its clinical implications.

In Table 4, a comparison of mean serum levels of calcium and vitamin D among different types of renal diseases is presented. An independent sample t-test reveals that patients with acute renal disease exhibit a significantly higher mean calcium level ($p = 0.009$) compared to those with chronic renal diseases.

Similarly, patients with acute renal diseases demonstrate significantly higher mean vitamin D levels ($p < 0.001$) than those with chronic renal disease.

Table 5 delves into the mean calcium and vitamin D levels of patients with renal diseases based on various characteristics. No significant differences are observed in mean calcium and vitamin D levels among age groups, sexes, smokers vs. non-smokers, users vs. non-users of dietary supplements, those undergoing medications vs. those not, and those on medications for <1 month vs. ≥1 month. However, patients who do not consume alcohol exhibit significantly higher mean calcium levels compared to those who drink 1 bottle ($p = 0.037$) and 3 bottles ($p = 0.049$). Vitamin D levels appear to decrease with increased alcohol consumption, showing statistical differences between those who do not drink and those consuming 3 bottles ($p = 0.015$). This suggests a potential impact of alcohol on vitamin D levels, warranting further investigation.

The findings underscore significant variations in calcium and vitamin D levels among different types of renal diseases and highlight associations with alcohol consumption, indicating the need for targeted interventions and closer examination of lifestyle factors in individuals with renal diseases.

Table1. Socio-Demographic Characteristics And Lifestyle Profile Of The Study Population

Characteristics	Control Group (n = 14) Mean ± SD or n (%)	Patients Living With Renal Disease (n = 32) Mean ± SD or n (%)
Age (years), Mean ± SD	41.07 ± 11.99	50.59 ± 14.88*
20-39 years	6 (42.9)	10 (31.2)
40-59 years	6 (42.9)	14 (43.8)
≥60 years	2 (14.3)	8 (25.0)
Sex		
Males	9 (64.3)	22 (68.8)
Females	5 (35.7)	10 (31.2)
Marital Status		
Married	8 (57.1)	24 (75.0)
Single	6 (42.9)	8 (25.0)
Occupation		
Employed	12 (85.7)	21 (65.6)
Unemployed	2 (14.3)	4 (12.5)
Retired	0 (0)	7 (21.9)
State of Origin		
Edo State	9 (64.3)	12 (37.5)
Delta State	4 (28.6)	9 (28.1)
Other States	1 (7.1)	11 (34.4)
Smoking Habit	14 (100)	13 (40.6)
No	0 (0)	19 (59.4)
Yes		
Extent of Smoking (n, 0 vs.19)	0 (0)	9 (47.4)
Half pack/day	0 (0)	7 (36.8)
One pack/day	0 (0)	3 (15.8)
2 packs/day		

Table 1 continue

Alcohol Consumption		
No	14 (100)	5 (15.6)
Yes	0 (0)	27 (84.4)
Extent of Alcohol Consumption (n, 0 v. 27)		
1 bottle of alcoholic beverage	0 (0)	12 (44.4)
2 bottles	0 (0)	12 (44.4)
3 bottles	0 (0)	3 (11.1)
Use of Dietary Supplements		
No	14 (100)	29 (90.6)
Yes	0 (0)	3 (9.4)
Regular Exposure to Sunlight		
No	0 (0)	0 (0)
Yes	14 (100.0)	32 (100)

Table 2.Clinical Characteristics Of The Patients Living With Renal Diseases

Characteristics	Frequency	Percentage
Type of Renal Disease		
Acute	18	56.2
Chronic	14	43.8
Ongoing Medications		
No	13	40.6
Yes	19	59.4
Duration of Medication		
< One Month	9	47.4
≥One Month	10	52.6
Difficulty in Movement		
No	23	71.9
Yes	9	28.1

Table 3. **Levels Of Calcium And Vitamin D Among Study Group**

Variables	Control (n = 14)	Patients with Renal Diseases (n = 32)	t-statistics	P – Value
Calcium	2.13 ± 0.37	1.96 ± 0.50	1.11	0.273
Vitamin D	71.47 ± 19.27	46.65 ± 29.69	2.87	0.006

Data are expressed as mean ± standard deviation, P<0.05(Significant), P>0.05(Non-significant).

Table 4. **Levels Of Calcium And Vitamin D Among Acute And Chronic Compared Renal Diseases**

Type of Renal Disease	Number of Patients	Calcium Level	Vitamin D Level
Acute	18	2.16 ± 0.36	63.22 ± 7.38
Chronic	14	1.70 ± 0.55	25.36 ± 33.96
Statistics (ANOVA)		*P = 0.009*	*P < 0.001*

Table 5. **The Mean Calcium and Vitamin D Levels of Patients Living With Renal Diseases Compared According To Some Of Their Characteristics**

Variables	N	Calcium Level (Mean ± SD; *P – Value)*	Vitamin D Level (Mean ± SD; *P – Value)*
Age			
20-39 years	10	1.86 ± 0.53	43.80 ± 28.85
40-59 years	14	2.13 ± 0.39	50.50 ± 22.49
≥60 years	8	1.79 ± 0.060	43.51 ± 42.71
		P = 0.229	*P = 0.821*
Sex			
Males	22	1.95 ± 0.52	51.40 ± 30.19
Females	10	1.99 ± 0.48	36.22 ± 26.83
		P = 0.863	*P = 0.183*
Smoking Habit			
None	13	2.01 ± 0.55	42.94 ± 32.58
Half	9	2.10 ± 0.36	51.48 ± 21.47
1 Pack	7	1.81 ± 0.51	40.30 ± 27.72
2 Packs	3	1.69 ± 0.72	63.10 ± 48.43
		P = 0.764	*P = 0.538*
Alcohol Consumption			
None	5	2.08 ± 0.54	46.64 ± 29.28
I bottle	12	2.28 ± 0.28	63.49 ± 24.92
2 bottles	12	1.73 ± 0.52	39.49 ± 27.71
3 bottles	3	1.46 ± 0.40	8.03 ± 1.70
		P = 0.011	*P = 0.014*
Use of Supplements			
No	29	1.93 ± 0.49	43.53 ± 26.49
Yes	2	2.22 ± 0.60	76.86 ± 47.61
		P = 0.368	*P = 0.062*
Ongoing Medication			
No	13	1.89 ± 0.52	53.97 ± 25.25
Yes	19	2.01 ± 0.50	41.65 ± 31.96
		P = 0.510	*P = 0.254*
Duration of Medication			
<1 month	9	2.07 ± 0.48	37.02 ± 28.17
≥1 Month	7	1.96 ± 0.53	45.82 ± 36.01
		P = 0.653	*P = 0.564*

Source: Field work, 2020.

DISCUSSION

Chronic kidney disease (CKD) stands as a potent predictor of early cardiovascular disease, presenting a burgeoning public health challenge. Hypovitaminosis D is increasingly associated with CKD progression and related cardiovascular consequences. Insufficient levels of the enzyme converting 25-(OH)vitamin D to its active form contribute to prevalent severe vitamin D insufficiency in CKD patients (Sandra, 2009). Vitamin D supplementation (ergocalciferol or cholecalciferol) shows promise in raising serum 25(OH) D levels, mitigating secondary hyperparathyroidism, and reducing bone fractures in CKD patients, irrespective of dialysis or transplantation status (Awodele *et al*., 2019). However, vitamin D supplementation does not significantly improve cardiovascular outcomes or reduce overall mortality in CKD patients.

A study in Edo State, Nigeria, reveals a predominantly male CKD population (68.8%), aged 40-59, married (75%), employed (65.6%), and originating from Edo state (37.5%). Recent data (Olusanya *et al*., 2022) report a 13.5% CKD prevalence in Nigeria, with a higher incidence in women (14.1%) compared to men (9.5%) which was not in agreement with the finding of the study that reported men to be higher which could attributed to small size in this study. These findings underscore the intricate interplay of CKD, vitamin D dynamics, and cardiovascular risks in diverse populations.

A 2019 study in the journal Nephrology, Dialysis, and Transplantation reported a 34% prevalence of chronic kidney disease (CKD) in individuals over 65, 12% in those aged 45-64, and 6% in the 18-44 age group (Holick,2004; Adewuyi,2019). Individuals above 80 faced a 75% higher CKD risk than those aged 65-74. Multiple chronic conditions, including diabetes, hypertension, and cardiovascular disease, heightened CKD risk, as did frailty and reduced fluid intake in adults over 65. Genetic changes, particularly in individuals over 65 with chronic conditions, were linked to increased CKD risk (KDIGO, 2017). Factors contributing to CKD include reduced kidney blood flow, alterations in kidney arteries, and decreased nephron count, as confirmed by various researchers (Nazario, 2005). Vitamin D deficiency, a global concern, affects 20-100% of the population, with smoking and alcohol consumption associated with lower vitamin D and calcium levels (Nazario,2015). The study revealed a prevalence of 56.2% for acute renal disease and 43.8% for chronic renal disease, aligning with findings in Lagos, Nigeria (Adewuyi *et al*., 2019), but differing from another study by Oguonu et al. (2020), potentially due to variations in sample size. These insights emphasize the multifactorial nature of CKD, ranging from age-related factors to lifestyle choices and genetic predispositions. Tejara 's study (2017), found a 51.0% incidence of acute renal injury and 14.11% of chronic kidney disease (CKD), aligning with the present study's acute kidney disease findings. Calcium levels in patients with renal disorders were not significantly different from healthy controls, but significantly lower mean vitamin D levels were observed in patients ($p = 0.006$). Vitamin D deficiency (VDD) is linked to acute and chronic kidney diseases, often resulting from impaired kidney activation of vitamin D and decreased intake due to dietary restrictions. Gonzalez (2017) discovered widespread 25 (OH)-VD deficiency in hemodialysis patients, and Bhan and Burnett-Bowie (2010) reported 79% of chronic hemodialysis patients with 25(OH)-VD levels of 30 ng/mL. VDD prevalence in stages 3 and 4 CKD patients, not on dialysis, was substantial, with only 29% and 17% having sufficient 25(OH)-VD levels, respectively (Bhan and Burnett-Bowie, 2010). While sunlight exposure is beneficial, it cannot fully correct vitamin D deficiency in renal disease, as the kidneys are crucial for converting the common form, 25-hydroxyvitamin D, to the active form, 1,25-dihydroxycholecalciferol.

Recommendations for minimal sunlight exposure apply, especially for those with reduced natural vitamin D synthesis (LaClair *et al*, 2005). Patients in CKD stages 1-5 with VDD or VDI should follow general population supplementation regimens (Ross *et al*., 2011;Chan and Johnson, 2022), emphasizing the critical role of vitamin D in renal health and the importance of tailored interventions. Recommendations on vitamin D supplementation vary; KDOQI suggests 1000-2000 IU/d for repletion, but CKD patients may require more intensive plans (KDOQI, 2022). For those over 65 with limited sun exposure, NICE recommends 400 IU of VD3 daily (NICE,2022). However, this excludes supplementation for individuals with deficiency [Bhan,2010]. Insufficient calcium and vitamin D are linked to increased CKD risk, and this study suggests potential benefits of vitamin D supplementation for CKD individuals (KDOQI, 2022).

CONCLUSION

Patients with renal illness had significantly lower vitamin D levels than healthy controls, although this had no effect on their calcium levels. Pre-dialysis CKD patients frequently present with hypovitaminosis D and hypocalcemia. In order to prevent hypervitaminosis and hypercalcimenia, renal illness patients receiving supplementation must have their vitamin D and calcium levels monitored regularly.

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Study Limitations

Reluctant of patients to disclose their health information led to usage of small size, insufficient fund and High cost of reagent were the limitation encounter in the study

Contribution

The study was conceptualized and draft by BIGA, GUO and OFO, reviewed by AEO, US and AUI and DU, UCO and KEI participated in collection, analysis and interpretation of data.

Conflict of Interest: None

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